ARGONNE NATIONAL LABORATORY **INTRA-LABORATORY MEMO**

Mar 10, 1998

To: J. Weinberger, ITD
Bld 201

From: F. Stevens

Subject: A new kind of antibody ANL-IN-98-026

CC: E. Huberman

The attached document, "Janusbody: A compact, bivalent designed protein consisting of a dimeric assembly of a single antibody domain" by F.J. Stevens andM. Schiffer is submitted for consideration as an Invention Report and potential patent application. I would encourage the Technology Transfer group to seriously consider aggressively marketing this invention, which if "owned" by a biotech company, would almost certainly attract significant venture capital.

Janusbody: A compact, bivalent designed protein consisting of a dimeric assembly of a single antibody domain.

F.J. Stevens
M. Schiffer
Center for Mechanistic Biology & Biotechnology
03/10/98

Antibodies comprise a diverse family of rather large proteins that have evolved as part of the natural defense mechanism of humans and other animals for protection against infection by bacteria, viruses, and other pathogens. These molecules consist of two sets of two different polypeptide chains of different size. The smaller of the two has a molecular weight of approximately 24,000 daltons while the larger has a molecular weight of at least 50,000 daltons. A typical antibody has a molecular weight on the order of 150,000 (and some types are slightly larger).

Each of the two subunits is subdivided into separately folded segments (of molecular weight approximately 12,000 daltons) known as domains. Various domains represent different functional constituents. The "business end" of the antibody consists of a binding site composed of contributions from the variable domains of the heavy and light chains (VH and VL, respectively). The term, variable, refers to the extensive amino acid sequence variation that is found in this domain when antibodies of different binding specificity are compared. This variability accounts for the diversity of antibody binding capabilities; it arises from the existence of multiple genes that encode these domains, and from mutations that are generated during the lifetime of each individual. In contrast, the "constant" domains that make up the rest of the antibody molecule show little variation and are, in fact, encoded by limited numbers of genes.

The ability of antibodies to recognize and bind selectively to an effectively unlimited range of molecules provides major commercial interest in their exploitation. For instance, the multibillion dollar immunodiagnostics industry uses antibodies for applications ranging from safety testing of blood units intended for transfusion or screening for cancer indicators to determining levels of particular hormones. Other medical applications include use as vectors for diagnostic imaging and agents for immunotherapy. As the expense associated with antibody development and production decreases, it can be anticipated that additional medical, as well as non-medical, applications will emerge. Non-traditional exploitations are very likely to include "diagnostic" systems for environmental monitoring or assurance of non-contamination of food as well as adaptations of antibodies as molecular vises or devices to hold and present other molecules in a defined orientation to increase the specificity or efficiency of particular steps in chemical manufacturing processes.

Reductions in cost have been obtained through the gradual shift away from polyclonal antisera obtained from animals to monoclonal antibodies (produced by mouse tumor cell lines) to recombinant antibodies produced by bacteria. In parallel, there has been an

evolution from the use of intact antibodies to the use of fragments such as an Fab which contains the VH and VL and one constant domain from each chain to an Fv which consists simply of VH and VL in combination (frequently with an artificial polypeptide linker to connect the two domains). To date, the Fv has been the smallest functional antibody construct.

The Janusbody will consist of two copies of the same protein subunit; hence, will require the bacterial expression of a single gene in contrast to the two genes or the two-gene-equivalent used to produce an Fv. However, as envisioned, the Janusbody will retain the full binding-interaction capacity contributed by the use of two protein subunits in the Fv, Fab, or intact antibody.

Isolated antibody variable domains spontaneously self-associate to form dimers consisting of two subunits. Until recently, crystallographic studies of these proteins indicated that the arrangement of the domains in the dimer closely resembled that found between the VH and VL in an antibody, Fab, or Fv. However, studies demonstrated that the dimers formed and dissociated rapidly with typical lifetimes on the order of one second. Thus, although the crystal structures of light chain dimers resemble that of the binding site of an antibody, the naturally occurring molecule is not amenable for development into useful binding reagents.

In the course of on-going studies, we constructed a recombinant form of a pathology-associated human VL. Unexpectedly the protein showed much stronger (~ 100–fold) dimerization than had been previously observed in any VL. Crystallographic analysis demonstrated an unexpected arrangement of domains in the dimer. Instead of the typical dimer motif, in which the complementarity determining segments (CDRs) responsible for interaction are arranged in a juxtaposed position, the domains in the high-affinity dimer were counterpoised. Each set of CDR segments (three per each domain) were positioned at opposite ends of the dimeric assembly. In this orientation they were paired with turns contributed by conserved "framework" portions of the domain resulting in a potential antigen binding surface comparable in size to that of an antibody.

Because of the 14 amino acid substitutions (out of 113 in the VL domain) that differentiated the atypical protein, the structural origin of the unusual properties was not evident. We have subsequently rationalized the observation. Of more significance, we have now developed the means to engineer this effect by introduction of amino acid substitutions at one or two sites in the domain and are confident that additional designs are possible.

This provides the means to develop a new class of antibody-like molecules in which binding sites are presented at opposite ends of a dimer of molecular weight ~24,000 daltons. We are provisionally designating these molecules "Janusbodies" combining the word "antibodies" the name of the mythical Roman deity "Janus" depicted with faces peering in opposite directions simultaneously.

Janusbodies should have numerous applications. Bivalence imparts two significant attributes. First, it provides the capability of cross-linking molecules to which it binds. Thus, these molecules might be used to mimic hormones whose functions are mediated by cross-linking specific cell surface receptors. Cross-linkage will also allow Janusbodies to be used to mediate agglutination of targeted molecules so that they may be removed from solution during filtration procedures. For viruses or bacteria that exhibit multiple copies of molecules on their surfaces, cross-linkers derived from a single clone will be sufficient. Smaller molecules can be aggregated by mixtures of Janusbodies that recognize independent sites on the molecule. In addition to possible medical application, this procedure might also have biotechnological applications. For instance, addition of specific Janusbodies to a bacterial cell extract containing a recombinant product would generate aggregates that could be separated from the remaining material by centrifugation, thus affecting in a single step both concentration and a high degree of purification. The target molecule and cross-linker would be separated by a simple second step (possibly by changing pH). It is expected that the Janusbodies could be reused in subsequent production runs.

The second advantage of bivalency is that it imparts a functional increase of affinity per unit mass. Effectively, when comparing a Janusbody to an Fv with an identical binding site the molecule with two binding sites has a higher probability of binding. Thus, the Janusbody, which intrinsically can be used in any current or future application of Fvs, can be used with equivalent effectiveness at lower concentration. Such applications include use in medical imaging procedures and a in broad range of immunodiagnostic products.

Implementation of Janusbody technology requires development of a system for selection of constructs of intended molecular recognition properties. It is expected that a straight-forward modification of the technique used for the production of single-chain Fvs through phage-display methodology will be adopted. For this purpose, an analog of the single-chain Fv genetic construct would be synthesized. Instead of a copy of a VH gene and a VL gene connected by a long linker peptide between the C-terminus of one domain and the N- terminus of the other, a short peptide would join the C-terminus of one VL to the N-terminus of a second VL gene. Although the prototype construct would contain two genes that encode the same amino acid sequence for both VLs, the synthetic genes would be different at the DNA level to maximize stability (i.e., prevent spontaneous cross-over). The Janusbody proxy displayed on the surface of phage would be functionally monovalent.

A "library" of Janusbody genetic constructs would be developed by use of PCR amplification during which the CDR segments of one VL gene and the FR turns of the second VL gene would be randomized. The resulting pool of genes encoding diverse intrinsic recognition capabilities would be incorporated into the phage in the same manner conventionally used to incorporate genes from humans or mice and subsequent selection would also be performed in the conventional manner. The performance of the resulting Janusbody would be optimized, if necessary, by subsequent rounds of random genetic variation and selction, or by protein engineering guided by crystallographic analyses of complexes formed with antigen.

Following selection, the DNA sequences of the two VL genes would be determined and a single gene construct incorporating the resulting CDR and FR components (that were originally dispersed between two gene segments) would be generated. The single gene construct would be inserted into a bacterial expression system and used for subsequent production of the Janusbody.

Work in this laboratory has demonstrated that Janusbody technology is feasible. The most technically challenging issue has already been resolved – that of developing simple, rational structural strategies to generate stable dimeric assemblies that display binding sites on opposing poles of the molecule.

The near-term commercial relevance of the Janusbody is directly demonstrated by the existing commercial interest in the development and exploitation of Fv constructs in various forms. The Janusbody should be able to directly replace the Fv in any application. Implementation of such replacements will be motivated by the potentially significant expenses in production of the reagent.

Potentially licensable products of a program to develop Janusbody technology include the libraries generated for selection of antigen-specific Janusbodies, as well as specific Janusbodies that are developed internally and by users of the Argonne Janus libraries.

INVENTION DISCLOSURE RECORD AND EVALUATION

ANL Case No. ANL-IN-98-026

The invention report appended hereto will serve to describe formally the invention.

1. Title of Invention: JANUSBODY: A COMPACT MOLECULE WITH ANTIBODY-ANTIGEN BINDING CHARACTERISTICS

2. Inventors:

Full Name: Fred J. Stevens
Residence Address: 548 Beaconsfield Ave.
City/State/Zip: Naperville, IL 60565
County: Will
Work Phone: 630-252-3837
Home Phone: 630-416-8015

Full Name: Marianne Schiffer
Residence Address: 4525 Downers Dr.
City/State/Zip: Downers Grove, IL
County: Du Page
Work Phone: 630-252-3883
Home Phone: 630-968-8578

3. Signature of Inventor and Date:	Signature of Witness and Date:
Fred J Stevens 5/11/98	Deborah K. Hanson 5/11/98
Marianne Schiffer 5/11/98	Priscilla Williams Stevens 5/11/98

(Witnesses are attesting they understand the description of the invention and that the signatures of the inventors are valid.)

4. Brief Description of Invention Emphasizing Unique and Novel Aspects (attach separate sheet if necessary):

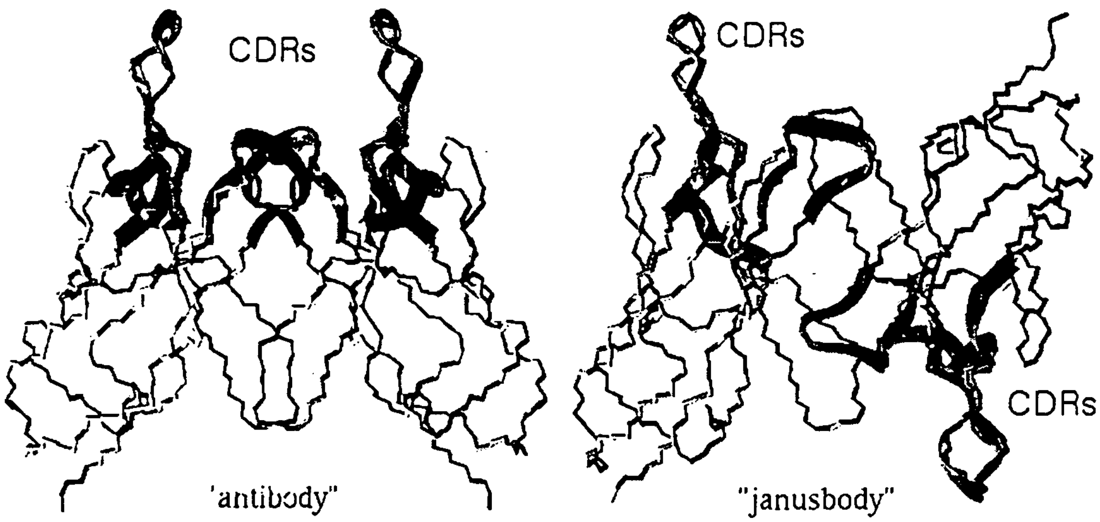
A classic antibody consists of several domains (independently folded protein substructures): 2 light chain variable domains, 2 light chain constant domains, 2 heavy chain variable domains, 6 or 8 heavy chain constant domains. In contrast, the Janusbody will consist solely of 2 light chain variable domains. Despite its small size, the Janusbody is, like an antibody, bivalent – capable of binding two identical target molecules.

The Janusbody is similar in size to a so-called single chain Fv (variable domain fragment) consisting of one light chain variable domain and one heavy chain variable domain. Substantial research and development into scFv molecules is currently being conducted, motivated by potential biotechnical and biomedical applications. Phage-display techniques are used to select scFvs of particular specificity for various applications. We expect that this approach will also be useful for the development of janusbodies for numerous applications.

We expect that production of janusbodies will be much more economical when compared to costs associated with production of scFv. This is a result of the fact that only one gene component (the light chain variable domain) will be involved rather than the two genes that must be co-expressed with an artificial linker segment to produce an scFv.

Yields of these molecules are typically very low, a fact that may reflect the low solubility of the heavy chain component of the scFv. Janusbodies consist of a single gene segment encoding a soluble, stable light chain variable domain.

Substantial work has also gone into making linked or bivalent scFvs for applications in which cross-linking of the target molecule is of importance. Janusbodies will be naturally bivalent. This allows cross-linking applications and also results in a two-fold increase in effective affinity per unit mass.



Janusbodies will be based on the light chain variable domain segments from human antibodies; thus they will be useful reagents for immunotherapy and immuno-imaging. They will not invoke immunological reactions associated with the use of antibodies of non-human origin for these purposes.

The above figure compares the domain arrangements in the typical light chain variable domain dimer (which emulates the conformation of an antibody) with that of a janusbody. The complementarity-determining regions (CDRs) are highlighted by ribbons. In the antibody, the CDRs from both domains are juxtaposed on the same surface of the molecule. In the janusbody, the CDRs are counterpoised on opposite poles of the dimer. It is anticipated that variation of the amino acid sequence of the CDRs and the adjacent loops (contributed by the framework segments) would lead to a family of janusbodies of different molecular binding specificities.

5. Dates and Places of Inventions:

Conception by Inventor March 8, 1998		At: ANL	
First Sketch or Drawing: n/a	At: n/a	In Workbook: n/a	Page: n/a
First Written Description: March 10, 1998	At: ANL	In Workbook: Memo to J. Weinberger	Page: n/a
Disclosure to Others:			
J. Weinberger (March 10, 1998)		At: ANL	
		At:	
		At:	
Completion of Model or Full Size Device: n/a		At:	
First Test or Operation of Invention: n/a		At:	

6. Has your invention been reduced to practice? In part . If yes, what was the performance of the invention?

We have demonstrated the ability to introduce specific amino acid variations that change the mode of self-association of human light chain variable domains such that a bivalent receptor (with binding sites at opposite poles of the molecule) can be constructed.

If no, what further development work is needed to reduce the invention to practice and who will fund it? (Include an estimate of the cost and manpower that will be needed.)

The major work anticipated is to introduce the Janusbody construct into a genetic system such that "libraries" of janusbodies can be screened for molecules of intended binding specificity. This procedure (phage display selection technology) is well-established for the selection of scFv molecules and no major technical hurdle is foreseen.

7. What are the potential uses of this invention by the government and/or by industry? What needs does it fulfill? What advantages does it have over existing products or processes?

The janusbody technology could replace conventional antibodies in all immunodiagnostic and immunodetection applications. Janusbodies would be the antibody reagent of choice in implementation of the massively parallel immunoassay described earlier (ANL-IN-97055; DOE S89,003). As a biotechnology tool, janusbodies could have substantial application as a purification tool for recombinant protein products. As a human protein, other novel applications could involve serum therapy; i.e., removal of toxins or other biological pathogens from blood.

The janusbody should be substantially more convenient to produce, and less expensive, than current antibody constructs.

8. List any industry contacts who have shown an interest in this invention or this line of work. Have you discussed the possibility of collaborative development through a CRADA? Did you use a non-disclosure agreement?

No discussion.

9. Have you disclosed this invention and/or what plans do you have to disclose aspects of this invention - publications, talks, public use? Please give the dates.

No outside disclosure of the janusbody concept. A paper describing the inverted domain configuration is in press. The manuscript does not specifically disclose the janusbody premise.

10. List other R&D organizations working in the technology area of your invention, including key names where possible.

No other R&D organization is likely to be working in this area.

11. Do you recommend foreign filing of this invention? If yes, what countries?

Yes. All developed nations.

12. List key words that may be useful in searching for prior art or competing technologies:

immunoassay; immunodiagnostics; immuno-imaging; scFv; phage-display; bivalent scFv